

Investigation of Vitamin C Content and Nutritional Values from the Fruits of *Citrus aurantium* L.

Khin Toe Toe Aung*, Ya Min Phyoe**

Abstract

The bitter orange fruit is one of the most widely consumed fruits in Myanmar. The bitter orange fruit is rich in antioxidants and vitamins. These vitamins promote a healthy immune system and prevent heart disease and high blood pressure. So the bitter orange fruit was chosen for chemical analysis. In this research work, bitter oranges were collected from Myoma market, Sinkaing Township, Mandalay Region. Firstly, the phytochemicals screening of bitter orange fruits were done by test tube method. And then, some nutritional values of *Citrus aurantium* L. were also examined. Finally, the vitamin C content was determined by iodimetric titration method.

Keywords : *Citrus aurantium* L., Phytochemical Screening, Nutrients, Vitamin C, Iodimetric titration method

Introduction

Bitter orange also called Seville orange, is known botanically as *Citrus aurantium* L. of the family Rutaceae. Bitter orange most likely originated in Southeast Asia and Myanmar was initially propagated in India and Persia. Bitter orange is known by many local common names in countries around the world.

The bitter orange tree is the source of numerous commercial products used in the fragrance and food industries. In the food industry, bitter orange oil, which is usually expressed from the fresh peels, is widely used as a flavoring agent. Bitter orange oil is used as flavoring for beverages, particularly liqueurs and to intensify the orange character of soft drinks (Mark Blumenthal, 2004).

Orange juices are a rich source of vitamin C. Oranges have several vitamins and minerals that our body needs to maintain a healthy heart. The major role of vitamin C is the prevention of scurvy; this causes the disease which leads to the formation of spots on the skin, spongy gums and bleeding from the mucous membranes (Website 1).

Vitamin C, has the chemical name ascorbic acid. It is water soluble vitamin. Although it is important for good health, humans do not have the ability to make their own vitamin C and must obtain it through diet or take it in vitamin supplements. Citrus fruits, potatoes and some green vegetables are known to be good sources of vitamin C (David A. Katz, 2013).

* Lecturer, Dr, Department of Chemistry, Yadanabon University

** Student, MSc., Department of Chemistry, Kyaukse University

Vitamins are organic compounds that have important biological functions. For instance, in humans they enable a variety of enzymes in the body to function. The human body cannot synthesize vitamin molecules, so they must be obtained in the diet. If a particular vitamin is lacking in the diet, a deficiency disease will result. It is not stable to heat, so cooking fruits and vegetables destroys much of their vitamin C content. It is also easily oxidized by certain compound, such as oxygen in the air. Therefore, the vitamin C content in an orange will be reduced if the orange has been cut in half and left exposed to the air for a period of time (Website 2, 3).

Botanical Description

Family	-	Rutaceae
Botanical name	-	<i>Citrus aurantium</i> L.
Myanmar name	-	Lein maw, Kabala
English name	-	Orange, Bitter orange
Part Used	-	Fruit



Figure 1. Plant and Fruits of *Citrus aurantium* L.

Citrus aurantium (bitter orange) is a plant belonging to the family Rutaceae. Orange is an ever green flowering tree. Height of orange tree is generally 9-10 m (although very old specimens have reached 15 m). Leaves alternate with winged-petioles, the blade ovate, bluntly toothed, emitting a strong citrus odour due to the presence of copious oil glands. Flowers axillary, borne singly or a few, white and very fragrant. Citrus fruit exceeds up to or occasionally 10 cm broad with thick skin containing sour and somewhat bitter pulp and several to numerous seeds within. This plant flowers during warmer months and fruits available later in the year. It is widespread throughout the Pacific and warm areas throughout the world.

Aim and Objectives

Aim

The aim of this research work is to determine the vitamin C content of bitter orange fruits using iodimetric method.

Objectives

- To collect the sample
- To carry out the phytochemical screening of the bitter orange fruits
- To determine the nutritional values
- To investigate the vitamin C (Ascorbic Acid) contents in bitter orange fruits

Materials and Methods

Chemicals and Apparatus

Chemicals

Distilled water, Ethanol, 1 % Hydrochloric acid, Pet-ether, 10 % aqueous sodium hydroxide, 10 % lead acetate, 1 % ferric chloride, 1 % potassium ferrocyanite, Benedict's solution, Chloroform, conc. sulphuric acid, Dragendroff's reagent, Acetic anhydride, Toluene, Potassium sulphate, Copper sulphate, Iodine, Potassium iodide, Ascorbic acid, Starch

Apparatus

Beaker, Conical flask, Test tube, Glass tube, Glass rod, Funnel, Porcelain crucible, Muffle furnace, Desiccator, Water bath, Soxhlet extractor, Condenser, 250 cm³ Round bottom flask, Kjeldahl's digestion flask, Burette, Volumetric flask, Pipette, Measuring cylinders, Analytical balances

Sample Collection

The samples of bitter orange fruits were collected from Myoma market, Sinkaing Township, Mandalay Region.

Preliminary Phytochemical Test for Bitter Orange Fruits by Test Tube Method

Preliminary phytochemical test for bitter orange fruits by test tube method are flavonoid, glycoside, polyphenol, reducing sugars, saponin, terpenoid and alkaloid test respectively.

Determination of Water Content in Bitter Orange Juice by Dean and Stark Method

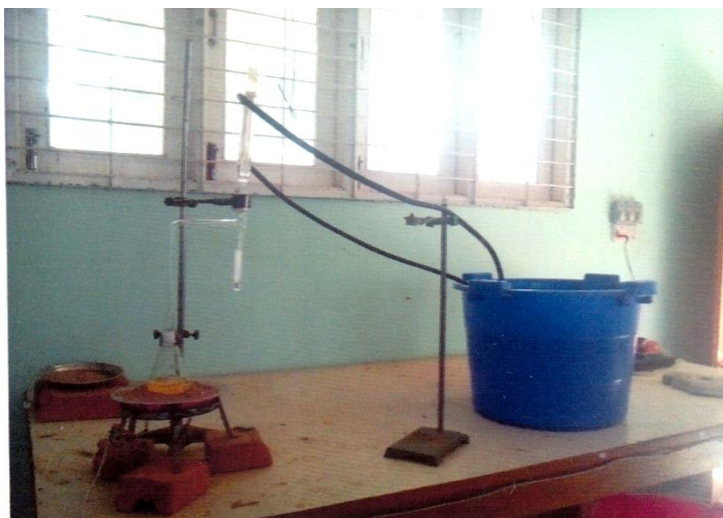
Bitter orange juice sample (10 cm³) and toluene (100 cm³) were placed in a clean dried round bottomed flask (250 cm³) and the Dean and Stark trap was attached to the flask. The mixture solution was heated and then distilled through the condenser. Toluene solution was upper layer in the trap and over flowed back into the distillation flask.

The distilled water was collected at the bottom of the graduated tube. After one hour distillation period, the heating was stopped. The volume of water was read off and the water percent in the sample was calculated from the following formula.

$$\text{Water content \%} = \frac{V \times 100}{W}$$

where, V = the volume of water (cm³) which was collected in the graduated tube

W = weight of sample



Determination of Nutritional Values of Bitter Orange Fruits

The nutritional values (ash, protein, fat and fibre) contents of bitter orange fruits were determined by AOAC method. The procedure was repeated until a constant weight was obtained.

Determination of Vitamin C (Ascorbic Acid) Content by Using Redox Titration

Preparation of Iodine Solution

Potassium iodide (KI) (2.0 g), iodine (1.3 g) and a few (cm^3) of distilled water were added into a 100 cm^3 beaker. The mixture was swirled for a few minutes until iodine was dissolved. Iodine solution was transferred to a 1 dm^3 volumetric flask, making sure to rinse all traces of solution into the volumetric flask using distilled water. The solution was made up to the 1 dm^3 mark with distilled water.

Preparation of Vitamin C Standard Solution

Vitamin C (ascorbic acid) (0.25 g) was dissolved in the beaker with 100 cm^3 distilled water. The solution was transferred into 250 cm^3 volumetric flask and dilute to the mark with distilled water.

Preparation of 1 % Starch Indicator Solution

Soluble starch (1 g) and 100 cm^3 of near boiling water were added into 100 cm^3 beaker. Then, it was stirred to well dissolve and allowed to cool before using.

Preparation of Sample Solution

The sample solution of bitter orange fruits was prepared. Firstly, the bitter orange fruits were peeled the skin off. The portions (100 g) were blended in a blender together with 10 cm^3 of distilled water. The juice was strained through the cheese cloth to remove the pulp. After straining, the fruit juice is mixed with distilled water to obtain 100 cm^3 of sample solution.

Standardization of the Iodine Solution with the Standard Vitamin C (Ascorbic Acid) Solution

Vitamin C solution (20 cm³) was pipetted into a 125 cm³ conical flask, 10 drops of 1 % starch solution were added and then titrated against iodine solution until blue-black colour observed. The titration was repeated at least three times. The results were tabulated in Table (3). Then, the concentration of iodine solution was computed.

Determination of Vitamin C (Ascorbic Acid) Content in Bitter Orange Fruits

Sample solution (20 cm³) was taken in a conical flask, 10 drops of 1 % starch solution were added and then titrated with standard iodine solution until blue-black colour appeared. The titration was repeated at least three times. The results were shown in Table (4). Then the amount of ascorbic acid content was calculated.

Results and Discussion

Preliminary Phytochemical Tests of Bitter Orange Fruits

Phytochemical tests of extract of bitter orange fruits were done. The results are tabulated in Table (1).

Table (1) Phytochemical Tests of the Bitter Orange Fruits

No.	Test	Extract	Test reagent	Observation	Results
1	Flavonoid	H ₂ O	10 % aqueous sodium hydroxide, dilute HCl	colorless solution	+
2	Glycoside	H ₂ O	10 % lead acetate	White ppt	+
3	Polyphenol	EtOH	1 % FeCl ₃ , 1 % K ₃ [Fe(CN) ₆]	Greenish blue color solution	+
4	Reducing sugar	H ₂ O	Benedict's solution	Reddish brown ppt	+
5	Saponin	H ₂ O	Distilled water	Formation of frothing	+
6	Terpenoid	H ₂ O	Chloroform, conc: H ₂ SO ₄	Red brown at the interface	+
7	Alkaloid	1 % HCl	Dragendroff's	Reddish brown ppt	+

(+) = presence, (-) = absence

According to the results of phytochemical tests, bitter orange fruits contained flavonoid, glycoside, polyphenol, reducing sugar, saponin, terpenoid and alkaloid respectively. Flavonoids and polyphenol are also powerful antioxidant agents. Glycosides and reducing sugar play numerous important roles in living organisms. Saponins also seem to help our immune system boost and to protect against viruses and bacteria. Terpenoid also have the ability to influence our brain neurons. Alkaloids may act as reservoirs for protein synthesis.



Nutritional Values in Bitter Orange Fruits

From the dried sample, the nutritive values, namely water content, ash, fibre, protein and fat contents were examined. The results are shown in Table (2).

Table (2) Nutritional Composition of Bitter Orange Fruits

No.	Items	% Composition
1	Water content	66.31
2	Ash	7.15
3	Protein	9.44
4	Fat	0.12
5	Fibre	10.48

In this table, nutrients in bitter orange fruits are plentiful and diverse. Protein is crucial to good health. The fruit is low in calories, contains no saturated fats or cholesterol, but is rich in dietary fibre. Fibre is to keep the digestive system healthy.

Standardization of the Iodine Solution with the Standard Vitamin C (Ascorbic Acid) Solution

Table (3) Titration of 20 cm³ Standard Ascorbic Acid with 0.0059 M Iodine Solution:

Indicator – Starch

No.	Initial volume (cm ³)	Final volume (cm ³)	Used volume (cm ³)
Rough	0	20	20
1	0	19.4	19.4
2	0	19.3	19.3
3	0	19.4	19.4
Mean volume			19.4



Determination of Ascorbic Acid Content in Bitter Orange Fruits

Table (4) Titration of 20 cm³ Fresh Juice of Bitter Orange with 6.5 cm³ of 0.0059 M

Iodine Solution

Indicator–Starch

No.	Initial volume (cm ³)	Final volume (cm ³)	Used volume (cm ³)
Rough	0	7	7
1	7	13.5	6.5
2	14	20.4	6.4
3	21	27.5	6.5
Mean volume			6.5



The vitamin C contents of bitter orange fruits were calculated. The result is shown in Table (5).

Table (5) Content of Vitamin C in Bitter Orange Fresh Juice

Sample	Ascorbic acid contents (mg/100 g)
Bitter orange	33.75

In this table, content of vitamin C in bitter orange fresh juice is 33.75 mg/100 g. Therefore, the fruit is an excellent source of vitamin C. Vitamin C is powerful natural antioxidant. Consumption of foods rich in vitamin C helps the body develop resistance against infectious agents and scavenge harmful, pro-inflammatory free radical from the blood.

Conclusion

In the research work, the bitter orange fruits were collected from Myoma market, Sinkaing Township, Mandalay Region. The preliminary phytochemical tests of the fruits of bitter orange were done. According to the phytochemical tests, the bitter orange fruits contained flavonoid, glycoside, polyphenol, reducing sugars, saponin, terpenoid and alkaloid respectively. Nutritional values of bitter orange fruit were found to be water content (66.31 %), ash (7.15 %), protein (9.44 %), fat (0.12 %) and fibre content (10.48 %) respectively.

Furthermore, the vitamin C (ascorbic acid) contents in bitter orange fresh juice were determined by Iodimetric Titration Method. The contents of vitamin C of bitter orange were found to be 33.75 mg per 100 g. Bitter orange fruit is not only an excellent source of vitamin C but also a good source of antioxidant. Therefore, bitter orange fruit should be consumed as raw and juice for the benefit of the health for all human beings.

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Online Materials

1. <https://www.healthline.com>nutrition>
2. <https://en.m.wikipedia.org>
3. <https://www.kazidomi.com>blog>

